Dispatches

Axon Degeneration: Where the Wld^S Things Are

Expression of the Wld^S protein significantly delays axon degeneration in injuries and diseases, but the mechanism for this protection is unknown. Two recent reports present evidence that axonal mitochondria are required for Wld^S-mediated axon protection.

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Axon degeneration is a pivotal pathological event in many acute and chronic neurological diseases. Remarkably, expression of the mutant Wallerian degeneration slow (Wld^S) protein strongly delays axonal degradation from various nerve injuries. Whereas wild-type axons begin to degenerate within hours of nerve transection, the distal segments of Wld^S axons remain structurally intact for weeks following the same injury, even long after the cell bodies have degenerated [1], indicating a degenerative process that is intrinsic to the axon and distinct from mechanisms governing cell body death [2,3]. The cellular targets of Wld^S activity that are responsible for conferring axonal protection, however, have remained a mystery. In this issue of *Current Biology*, two separate studies provide evidence that Wld^S enhances physiological functions of the mitochondria and show that axonal mitochondria are required for Wld^S-dependent axon protection [4,5].

Wld^S is a remarkable protein that significantly delays axonal degeneration from nerve injuries and diseases [6]. It is the product of a chimeric transcript consisting of the amino-terminal 70 amino acids of a ubiquitination factor (*E4b*) and the full functional sequence of a nicotinamide adenine dinucleotide (NAD) synthetic enzyme (*NMNAT1*) [7]. Expression of this fusion gene product *in vitro* and *in vivo* is sufficient to confer axon protection to wild-type axons. This protection is conserved across many species, including rodents and *Drosophila* [8,9]. Expression of the normally nuclear Nmnat1 enzyme in extranuclear compartments is sufficient to confer axon protection [10,11], and deletion of the Nmnat enzymatic domain in Wld^S abolishes axon protection [12,13], suggesting that extranuclear enzymatic activity of Nmnat is necessary and sufficient to promote axonal survival. But how Wld^S/Nmnat protein activities affect the normal axonal degeneration process has been a mystery.

To help identify additional molecular players critical for maintaining axonal survival, Fang *et al.* [5] screened 1,400 existing enhancer- and promoter-containing *P*-element (EP) lines in *Drosophila* to search for candidates that, when upregulated, can delay degeneration of axons after axotomy. To achieve this, the authors developed a novel axonal injury paradigm that allows for easy visualization and quantification of axonal degeneration. By using the Gal4–UAS system to express a GFP reporter under the control of the *dpr*pGaw–Gal4 driver, which is expressed by a subset of sensory neurons along *Drosophila* wings, the authors could conveniently assay for axon degeneration by simply severing the wings and monitoring GFP fluorescence along the peripheral wing nerves.

One candidate that the authors identified from the screen was dNmnat, a *Drosophila* homologue of mammalian Nmnat. Upregulation of dNmnat strongly delayed axon degeneration after axotomy while knockdown of endogenous dNmnat by RNA interference (RNAi) led to spontaneous degeneration [5], showing that dNmnat is necessary and sufficient for axonal survival after injury. This is consistent with the previously reported role of Nmnat2, a mammalian Nmnat isoform, as an intrinsic axonal survival factor [14]. Moreover, the axonal degeneration resulting from loss of *Drosophila* dNmnat could be rescued by expression of Wld^s and all mammalian Nmnat isoforms [5], suggesting that the activity of Wld^S as well as ectopic Nmnat is conserved and protects axons by substituting for the endogenous axonal trophic property of dNmnat.

What might be the mechanism and cellular target of dNmnat- and Wld^S-mediated axonal protection? Insight into this issue came when Fang *et al.* [5] examined organelle dynamics in injured axons and observed that there was a concomitant decrease in the levels of mitochondria, as evidenced by decreased expression of mitochondria-targeted GFP, in the distal axon after axotomy as well as after knockdown of dNmnat [5]. Interestingly, this depletion of axonal mitochondria, which was not simply due to decreased availability of microtubule transport proteins, was fully rescued by upregulation of dNmnat, raising the possibility that dNmnat may function to stabilize the mitochondria and preserve their physiological functions in the axon (Figure 1).

What are the specific effects of Wld^S/Nmnat activity on mitochondria, and are these mitochondrial events responsible for conferring axonal protection by the Wld^S/Nmnat proteins? Findings from the second study by Avery *et al.* [4] provide a clue. Based on previous reports and the authors' own observations that the majority of extranuclear Wld^S proteins are localized in the mitochondria [4,15], Avery *et al.* [4] hypothesized that Wld^S or Nmnat activity within the mitochondria may alter mitochondrial dynamics or functions to promote axonal survival. To test this, the authors performed laser axotomy on the peripheral axons of *Drosophila* larvae and found that, although mitochondrial movement was markedly diminished in the distal segments of wild-type axons after injury, mitochondrial motility was remarkably preserved in the injured Wld^S axons. In fact, despite having a similar number and size of mitochondria, uninjured Wld^S axons exhibited a significantly higher number of motile mitochondria than uninjured wild-type axons [4].

As Ca^{2+} induces undocking of mitochondria from the kinesin-dependent anterograde

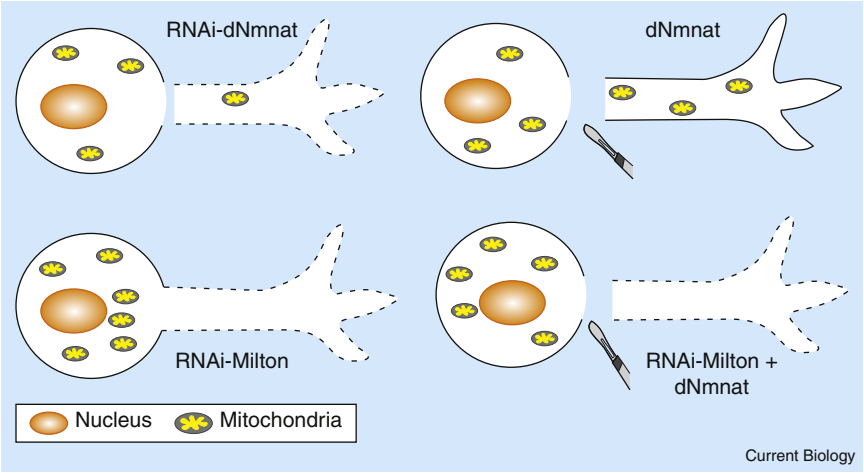

Figure 1. A model of mitochondria-dependent axon protection.

The *Drosophila* protein dNmnat is necessary and sufficient for axonal survival, as RNAi-mediated knockdown of endogenous dNmnat induces spontaneous axon degeneration and depletion of mitochondria in the axon (top left), while upregulation of dNmnat delays axon degeneration from axotomy and rescues the depletion of axonal mitochondria (top right). Blocking mitochondrial entry into the axon by knocking down Milton, a linker protein required for anterograde mitochondrial transport, leads to spontaneous axon degeneration (bottom left). This degeneration cannot be rescued even with upregulation of dNmnat (bottom right), indicating that axonal mitochondria are required for dNmnat-mediated maintenance of normal axon survival and axon protection following nerve injuries.

transport machinery [16], Avery *et al.* [4] further reasoned that the difference in mitochondrial motility between wild-type and Wld^S axons may reflect underlying differences in the levels of axonal Ca^{2+}. Indeed, measuring intra-axonal Ca^{2+} levels after laser axotomy revealed a rapid Ca^{2+} spike in the injured wild-type axons, but this was significantly attenuated in Wld^S axons. Moreover, the suppression of the post-injury Ca^{2+} spike in Wld^S axons was dependent on enzymatic activity of Nmnat, as only the Nmnat isoforms that retain enzymatic activity showed attenuation of the injury-induced Ca^{2+} spike [4].

What can account for the differences in mitochondrial motility and Ca^{2+} levels between wild-type and Wld^S axons? As mitochondria are capable of buffering Ca^{2+} [17], it is possible that Wld^S or Nmnat activity may directly enhance mitochondrial buffering of cytoplasmic Ca^{2+} and thereby decrease Ca^{2+} levels in the axon. Moreover, as more Ca^{2+} is buffered by the mitochondria, less Ca^{2+} may be available in the axoplasm to undock mitochondria from microtubule-based transport, thereby increasing the overall number of motile mitochondria. In support of this idea, when the authors compared the Ca^{2+} loading capacity of purified wild-type and Wld^S mammalian mitochondria, they found that the Wld^S mitochondria exhibited greater intrinsic capacitance for Ca^{2+} than that of wild-type mitochondria, suggesting that Wld^S/Nmnat activity can directly enhance mitochondrial handling of Ca^{2+} [4] (Figure 2).

Importantly, do these mitochondrial dynamics contribute to axonal survival, and are the axonal mitochondria necessary targets for Wld^S/Nmnat-mediated axonal protection? To address these questions, both Fang *et al.* [5] and Avery *et al.* [4] used a clever method to suppress mitochondrial entry into the axon. Previously, it was found that mutations in Miro or Milton proteins, which are linker proteins required for kinesin-dependent anterograde transport of mitochondria, exclude mitochondria from the axon [18,19]. By taking advantage of this finding, the authors could effectively retain mitochondria in the soma and block entry of the organelle into the axon by decreasing protein levels or by perturbing normal functions of Miro or Milton.

Interestingly, when anterograde mitochondrial movement and entry into the axon are attenuated, both studies showed that the axonal protection by Wld^S or Nmnat upregulation was also greatly diminished. For instance, sequestration of mitochondria in the cell body by RNAi-mediated knockdown of Milton resulted in spontaneous, progressive degeneration of the axon, which could not be rescued by overexpression of Wld^S or dNmnat [5]. Similarly, expression of a mutant allele of Miro

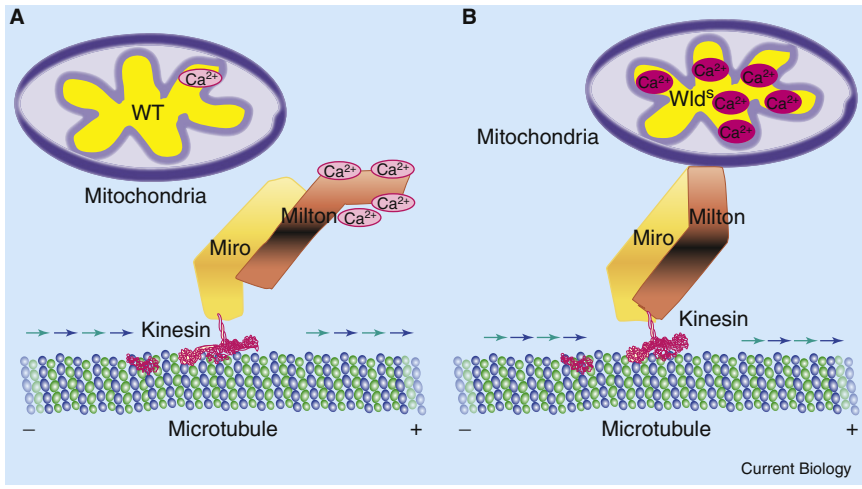

Figure 2. Wld^S activity in mitochondrial dynamics and function.

(A) In injured wild-type (WT) axons, an influx of extracellular Ca^{2+} exceeds the threshold of mitochondrial Ca^{2+} buffering and leads to a local, transient increase of Ca^{2+} in the axon. Ca^{2+} binding to the Milton–Miro protein complex leads to undocking of mitochondria from the kinesin-dependent anterograde transport machinery, thereby decreasing mitochondrial movement. (B) In injured Wld^S axons, increased Ca^{2+} buffering by mitochondria decreases available free Ca^{2+} to bind to the Milton–Miro complex, thus maintaining the attachment of mitochondria to the anterograde transport machinery and preserving motility of mitochondria in the axon.

was sufficient to dominantly suppress Wld^S-mediated axonal protection [4]. Both studies therefore showed that expression of Nmnat or Wld^S proteins failed to protect axons after injury when Miro or Milton function was compromised, indicating that the presence of mitochondria in the axon is required for Nmnat/Wld^s-mediated axonal protection (Figure 1). However, as it is unclear whether the transport of other organelles and proteins is also disrupted with decreased Miro/Milton function, it will be critical to differentiate whether the loss of Wld^S protection is due to decreased mitochondrial numbers and function in the axon, or simply to decreased transport or expression of the Wld^S protein in the axon.

The two reports together demonstrate that Wld^S/Nmnat activity enhances mitochondrial motility and Ca^{2+} buffering and that the mitochondrion is an organelle necessary for Wld^S/Nmnat-mediated axonal protection. The processes regulating axon degeneration and the Wld^S/Nmnat enzymatic activities that are critical for axonal protection thus converge at axonal mitochondria. A clear future direction is to address whether directly enhancing these mitochondrial functions is sufficient to exert axonal protection. Moreover, identifying whether known enzymatic metabolites of the Wld^S/Nmnat proteins, such as NAD^+, interact with molecules in the mitochondria will be instrumental in understanding the full downstream mechanisms of Wld^S/Nmnat-mediated axon protection. Although there is still much to learn about the molecular processes regulating axonal degeneration and survival, these two reports have given us a boost by placing the focus squarely on the axonal mitochondria.

References

1. Deckwerth, T.L., and Johnson, E.M., Jr. (1994). Neurites can remain viable after destruction of the neuronal soma by programmed cell death (apoptosis). Dev. Biol. *165*, 63–72.
2. Wang, J.T., Medress, Z.A., and Barres, B.A. (2012). Axon degeneration: molecular mechanisms of a self-destruction pathway. J. Cell Biol. *196*, 7–18.
3. Coleman, M.P., and Freeman, M.R. (2010). Wallerian degeneration, wld(s), and nmnat. Annu. Rev. Neurosci. *33*, 245–267.
4. Avery, M.A., Rooney, T., Wishart, T.M., Pandya, J.D., Gillingwater, T.H., Geddes, J.W., Sullivan, P., and Freeman, M.R. (2012). Wld^S prevents axon degeneration through increased mitochondrial flux and enhanced mitochondrial Ca^{2+} buffering. Curr. Biol. *22*, 596–600.
5. Fang, Y., Soares, L., Teng, X., Geary, M., and Bonini, N. (2012). A novel *Drosophila* model of nerve injury reveals an essential role of Nmnat in maintaining axonal integrity. Curr. Biol. *22*, 590–595.
6. Lunn, E.R., Perry, V.H., Brown, M.C., Rosen, H., and Gordon, S. (1989). Absence of Wallerian degeneration does not hinder regeneration in peripheral nerve. Eur. J. Neurosci. *1*, 27–33.
7. Mack, T.G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., *et al*. (2001). Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat. Neurosci. *4*, 1199–1206.
8. Hoopfer, E.D., McLaughlin, T., Watts, R.J., Schuldiner, O., O'Leary, D.D., and Luo, L. (2006). Wlds protection distinguishes axon degeneration following injury from naturally occurring developmental pruning. Neuron *50*, 883–895.
9. MacDonald, J.M., Beach, M.G., Porpiglia, E., Sheehan, A.E., Watts, R.J., and Freeman, M.R. (2006). The Drosophila cell corpse engulfment receptor Draper mediates glial clearance of severed axons. Neuron *50*, 869–881.
10. Beirowski, B., Babetto, E., Gilley, J., Mazzola, F., Conforti, L., Janeckova, L., Magni, G., Ribchester, R.R., and Coleman, M.P. (2009). Non-nuclear Wld(S) determines its neuroprotective efficacy for axons and synapses in vivo. J. Neurosci. *29*, 653–668.
11. Sasaki, Y., Vohra, B.P., Baloh, R.H., and Milbrandt, J. (2009). Transgenic mice expressing the Nmnat1 protein manifest robust delay in axonal degeneration in vivo. J. Neurosci. *29*, 6526–6534.
12. Avery, M.A., Sheehan, A.E., Kerr, K.S., Wang, J., and Freeman, M.R. (2009). Wld S requires Nmnat1 enzymatic activity and N16-VCP interactions to suppress Wallerian degeneration. J. Cell Biol. *184*, 501–513.
13. Conforti, L., Wilbrey, A., Morreale, G., Janeckova, L., Beirowski, B., Adalbert, R., Mazzola, F., Di Stefano, M., Hartley, R., Babetto, E., *et al*. (2009). Wld S protein requires Nmnat activity and a short N-terminal sequence to protect axons in mice. J. Cell Biol. *184*, 491–500.
14. Gilley, J., and Coleman, M.P. (2010). Endogenous Nmnat2 is an essential survival factor for maintenance of healthy axons. PLoS Biol. *8*, e1000300.
15. Yahata, N., Yuasa, S., and Araki, T. (2009). Nicotinamide mononucleotide adenylyltransferase expression in mitochondrial matrix delays Wallerian degeneration. J. Neurosci. *29*, 6276–6284.
16. Wang, X., and Schwarz, T.L. (2009). The mechanism of Ca2+-dependent regulation of kinesin-mediated mitochondrial motility. Cell *136*, 163–174.
17. MacAskill, A.F., and Kittler, J.T. (2010). Control of mitochondrial transport and localization in neurons. Trends Cell Biol. *20*, 102–112.
18. Glater, E.E., Megeath, L.J., Stowers, R.S., and Schwarz, T.L. (2006). Axonal transport of mitochondria requires milton to recruit kinesin heavy chain and is light chain independent. J. Cell Biol. *173*, 545–557.
19. Stowers, R.S., Megeath, L.J., Gorska-Andrzejak, J., Meinertzhagen, I.A., and Schwarz, T.L. (2002). Axonal transport of mitochondria to synapses depends on milton, a novel Drosophila protein. Neuron *36*, 1063–1077.

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DOI: 10.1016/j.cub.2012.02.056

Visual Perception: Knowing What to Expect

If perception is hypothesis, where do the hypotheses come from? A new study suggests that the human visual system uses the history of past stimulation to predict its current input.

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It is often said that we live in a changing world. As we go through life we adapt to those changes and build up expectations of what the future will hold. Our sensory systems face a similar challenge in dealing with different environments. There are many examples of sensory systems that are in some fashion optimised to their natural environment: consider, for example, the large eyes of the nocturnal bush baby or the acute sense of smell of the foraging honey bee. Sensory adaptation can be viewed as a process by which our sensory systems tend to remain optimized to a changing environment. Under this view, sensory systems are adaptive systems perhaps sharing principles of operation with systems as diverse as ant colonies and economies. In his classic book *Adaptation in Natural and Artificial Systems*, Holland [1] poses several fundamental questions for the study of adaptive systems. "What part of the history of its interaction with the environment does the organism retain?" is of key importance as it asks what knowledge drives the system to adapt. This question is directly addressed in the